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MRID No. 422506-01

DATA EVALUATION RECORD

1. **CHEMICAL:** Bromoxynil Octanoate.
Shaughnessey No. 035302.

2. **TEST MATERIAL:** 1) Nonradiolabeled Bromoxynil Octanoate Technical; M & B Lot No. CN-51033; 97.2% active ingredient; a waxy brown solid. 2) ^{14}C-Bromoxynil Octanoate in toluene; Lot No. 011H9209; 39.4 mCi/mmole; >98% radiopurity.

3. **STUDY TYPE:** Estuarine Fish Acute Flow-Through Toxicity Test. Species Tested: Sheepshead Minnow (*Cyprinodon variegatus*).

4. **CITATION:** Machado, M.W. 1992. (Bromoxynil Octanoate Technical) - Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*) Under Flow-Through Conditions. SLI Report No. 91-7-3853. Performed by Springborn Laboratories, Inc., Wareham, MA. Submitted by Rhone-Poulenc Ag Company, Research Triangle Park, NC. EPA MRID No. 422506-01.

5. **REVIEWED BY:**

Rosemary Graham Mora, M.S.
Associate Scientist
KBN Engineering and
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Signature: Louis M Rifici for RGM
Date: 6/25/93

6. **APPROVED BY:**

Louis M. Rifici, M.S.
Associate Scientist
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Signature: Louis M Rifici
Date: 6/25/93

Henry T. Craven, M.S.
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Signature: Michael Davy 12-1-92
Dennis Bleim 12-16-92
Date: Henry T. Craven 3/23/93

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an acute flow-through toxicity study using estuarine fish. Based on mean measured concentrations, the 96-hour LC_{50} for *Cyprinodon variegatus* exposed to Bromoxynil Octanoate Technical was 0.17 mg a.i./l. Therefore, Bromoxynil Octanoate Technical is classified as highly toxic to sheepshead minnow. The NOEC could not be determined since mortality was noted at all test concentrations.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** The sheepshead minnow (*Cyprinodon variegatus*) were obtained from Aquatic Biosystems, a commercial supplier in Fort Collins, CO. The fish were maintained in a 500-l tank under recirculating conditions and a photoperiod of 16 hours of light and 8 hours of darkness. The water in the culture tank had a temperature of 21-22°C, a pH of 7.5, and a salinity of 31-32 parts per thousand (ppt). The fish were maintained under these conditions for a minimum of 14 days prior to test initiation. The fish were fed a commercial flake food, *ad libitum*, daily except for the 48 hours prior to test initiation.

No mortality was observed in the 48-hour period prior to test initiation. A representative sample of the test fish population had a mean wet weight of 0.39 (0.15-0.83) g and a mean total length of 29 (22-39) mm.

B. **Test System:** The test system consisted of a constant-flow serial diluter (dilution factor of 60%), a temperature-controlled water bath, and 14 glass aquaria (39 x 20 x 25 cm). The solution volume was 11 l and the solution depth was 14.5 cm. The flow rate of test solution to each aquarium provided 6.5 volume replacements/day. The photoperiod during the test was the same as that during holding with a light intensity of 20-70 footcandles (216-756 lux). Sudden transitions from light to dark and dark to light were avoided.

The dilution water was filtered (5 and 20 µm), natural seawater from Cape Cod Canal, Bourne, MA and was the same as that used during holding. The water had a salinity of 30-31 ppt and a pH of 7.7-7.9.

The radiolabeled test material was prepared for testing by evaporating the toluene solvent with a nitrogen stream. A radiolabeled superstock solution (0.403 mg a.i./ml) was prepared by combining the radiolabeled test material with acetone to a final volume of 100 ml. A 23.0 ml portion of this superstock was combined with 2669 mg (2595 mg a.i.) of non-labeled test material and diluted with acetone to 250 ml, resulting in a final stock solution of 10.42 mg a.i./ml.

C. **Dosage:** Ninety-six-hour flow-through test. Based on the results of preliminary testing, five nominal concentrations were used (0.13, 0.22, 0.36, 0.60, and 1.0 mg a.i./l). In addition, a dilution water control and a solvent control (0.4 ml acetone/ml) were included.

D. **Design:** Ten fish were impartially loaded into each of two replicate aquaria (twenty fish/treatment level). The organism loading rate was 0.054 g/l/day. The fish were not fed during the test.

Biological observations and observations of physical characteristics of the test solutions were noted at test initiation and every 24 hours thereafter. Mortality was defined as lack of movement by exposed organisms. Dead fish were removed at each observation.

Dissolved oxygen, pH, salinity, and temperature were measured daily in each replicate chamber. The temperature in the B replicate of the lowest test concentration (0.13 mg a.i./l nominal) was monitored continuously using a min/max thermometer.

Chemical analysis of ^{14}C-Bromoxynil Octanoate was performed using liquid scintillation counting on each test solution collected on days 0 and 4 to verify the test concentrations.

E. **Statistics:** The 96-hour LC_{50} value and 95% confidence interval was determined using a computer program developed by Stephan (1977, 1982).

12. **REPORTED RESULTS:** The mean measured concentrations were 0.16, 0.20, 0.43, 0.46, and 0.68 mg a.i./l (Table 2, attached). The coefficients of variation averaged 17% for all mean measured concentrations. "Throughout the pretest and definitive exposures, undissolved test material (i.e., film on solution's surface) was observed as a precipitate at the tip of the syringe tubing and as a film on the solution's surface in the solution contained in the diluter's mixing chamber."

The mortality and observation data demonstrated 85-100% mortality at concentrations $\geq$0.20 mg a.i./l and 25% at 0.16 mg a.i./l. No mortality was observed in the controls (Table 3, attached).

The 96-hour LC_{50} for *Cyprinodon variegatus* exposed to Bromoxynil Octanoate Technical was 0.17 mg a.i./l mean

measured concentration with a confidence interval of 0.16-0.19 mg a.i./l. The slope of concentration-response curve was 7.4228. Sublethal effects or mortality were observed at all test concentrations. The NOEC was <0.16 mg a.i./l mean measured concentration.

During the study, the temperature was 22-24°C, the pH was 7.6-7.9, and the dissolved oxygen concentration was 4.9-7.0 mg a.i./l (68-97% of saturation). The continuous temperature of the test solution ranged from 23 to 24°C. The salinity was 30-31 ppt.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "Based on US EPA (1985) criteria, Bromoxynil Octanoate Technical would be classified as highly toxic to sheepshead minnow."

A Good Laboratory Practice Compliance Statement and a Quality Assurance Unit Statement were included in the report, indicating that the study was in accordance with GLP regulations (40 CFR, Part 160).

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedures were generally in accordance with the SEP, except for the following deviations:

— The test salinity was 30-31 ppt; the SEP recommends a salinity of 10-17 ppt for estuarine fish.

The test organisms were impartially selected and distributed to the test chambers. The SEP recommends random assignment of test organisms to test chambers.

The report does not indicate the age of the test organisms.

The highest concentration of solvent used in this study was reported as 0.4 ml/ml; the SEP recommends $\leq$0.1 ml/l in flow-through studies. According to the reviewer's calculation from information provided on solution preparation and flow rates of stock solution and dilution water, the maximum concentration would be 0.096 ml/l. This is a discrepancy in the report.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal computer program to determine the 96-hour LC_{50} value and its 95% confidence interval (printout, attached). and obtained the same results as the author.

C. **Discussion/Results:** This study is scientifically sound and meets the guideline requirements for an acute flow-through toxicity study using estuarine fish. Based on mean measured concentrations, the 96-hour LC_{50} was 0.17 mg a.i./l which classifies Bromoxynil Octanoate Technical as highly toxic to sheepshead minnow (*Cyprinodon variegatus*). The NOEC could not be determined from this study since mortality was observed at all concentrations.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** N/A.

(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, June 1, 1992.